MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *CAPOETA TRUTTA* (CYPRINIDAE) AND *PLANILIZA ABU* (MUGILIDAE) FRESHWATER FISH IN SULAIMANI GOVERNORATE, IRAQ

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Abstract. The morphological similarities of species of *Capoeta* and *Planiliza* genera make the identification difficult in some cases. Thus, this classification should be confirmed by molecular examination. In this study, 150 specimens of *Capoeta trutta* (Cyprinidae) were collected from Dukan Lake and 120 specimens of *Planiliza abu* (Mugilidae) from Sirwan River in northwestern and southeastern Sulaimani Governorate, respectively. The two fish species recorded were native species. The DNA sequences of both species of fish (*C. trutta* and *P. abu*) were mitochondrial DNA cytochrome c oxidase subunit I (mtDNA COI) locus 617 bp with 61 cytochrome b (cytb) gene, partial cds; mitochondrial, 446 bp respectively. Following analysis the sequences were compared with sequences of other genera and fish species stored in GenBank. DNA sequencing results showed that studied species belong to *Capoeta trutta* and *Planiliza abu*. We conclude, in the view of the results of the present study, that DNA sequence analysis revealed and confirmed the validity of these two species.
Keywords: *fishes, morphometric measure and meristic, molecular examination, DNA sequencing, Iraq*

Introduction

Among the several families of freshwater fishes in the world the most diverse one is Cyprinidae having 220 genera including 2420 species, which belong to Cypriniformes order (Nelson, 2006). Most of Iraqi fish belong to Cyprinidae family, which involving 16 genera with 32 cyprinid species (Coad, 1998; Coad and Hussein, 2007). The distribution area of cyprinid genus *Capoeta* includes Western to Central Asia, such as Armenia, Azerbaijan, Afghanistan, Israel, Anatolia, Iraq, Uzbekistan, Georgia, and Iran (Banarescu, 1991). Genus *Capoeta* includes almost 10 species, 4 out of these occur in Iraq (Coad, 2010). The species commonly appear in streams and lakes, thus in both fast and slow flowing waters (Geldiay and Balik, 1996). *Capoeta trutta* (Heckel, 1843) is a fish species having economic importance with wide distribution in Turkey, Iran, Iraq and Syria (Gunduz et al., 2014), which is dominantly thriving in both the Euphrates and Tigris river systems (Geldiay and Balik, 2007).

The grey mullets or mullets were discovered world-wide in temperate to tropical coastal waters directly entering in estuaries and they are also resident in freshwaters. There are around 75 species and 20 genera in world (Nelson et al., 2016). In Iraq only four species are exist (Coad, 2010). *Planiliza abu* is a mugilid species discovered in

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channels, drains, lakes, reservoirs ponds, canals, rivers, and streams on fish farms with entering estuaries. Ozdilek (2003) and Kuru (1979) state that in Syria, Iraq, Pakistan, Turkey and Iran, mullet often occurs in inhabited places or schools. *Liza abu*, mugilid fish (Heckel, 1843), locally known as khisni, is distributed in all part of mid and south inland waters of Iraq (Al-Daham, 1984).

Fishes in the genera *Chelon*, *Ellochelon* and *Planiliza* were previously in the genus *Liza* (Jordan and Swain, 1884). Molecular data has caused a re-assessment that can be found in the literature (Durand et al., 2012*;* Nematzadeh et al., 2013; Xia et al., 2016). Various authors have or have not accepted, these generic replacements but the review of these opinions is beyond the scope of this paper. Most of the literature cited below refers to the species discussed under the genus *Liza* (except for *Mugil cephalus*) but the genus name has been changed in this text for consistency.

Nowadays via using characteristics other than morphological traits mullet species phylogenic relationships were determined based on molecular genetics and advanced techniques have been developed for studying DNAs in diverse populations and also for identifying fish species via using nuclear (nDNA) and mitochondrial (mtDNA) genomes (Semina et al., 2007; Avis, 1991; Papasotiropoulos et al., 2007). For the study of phylogenetic relationships and molecular systematic in population genetics the mtDNA is an efficient genetic marker due to maternal inheritance, and lesser mean rate of recombination, of replacement and exchange in mtDNA nucleotides than those in nDNA (Ghorashi et al., 2008; Asensio, 2007).

The morphological similarities of species of *Capoeta* and *Planiliza* genera make the identification difficult in some cases. Thus, the aim of this study is to confirm this classification by molecular examination.

Materials and methods

Description of study area

Dukan Lake is situated in north western Sulaimani City, Kurdistan Region, in the north of Iraq. It is approximately 76 km far from the city center (Shaban, 1980). According to Toma (2000) the lake has unregulated spillway at 515 m above sea level and full-pool operating altitude of 511 m, its boundaries extend between 34°17'N – 36°33'N latitude with 43°17'E – 46°24'E longitude (*Fig. 1*).

Sirwan River is situated in south eastern Sulaimani City. It is called Diyala in Arabic and Sirwan in Kurdish. It flows in western Iran from its headwaters in Zagros Mountains to south of Baghdad to its ultimate confluence with the Tigris River. The latitude of Sirwan River is 33° 13' 14.88" N and its longitude is 44° 30' 23.04" E (*Fig. 1*).

Sample collection

A total of 150 *Capoeta trutta* specimens have been collected from Dukan Lake in north western Sulaimani governorate and 120 individual of *Planiliza abu* were collected from Sirwan River in the Sulaimani governorate by fishermen using gill nets, during the period from November, 2017 until May 2018. Fish were transported with local river water in a cool box to laboratory.

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Figure 1. *Map of Iraq showing Dukan Lake and Sirwan River*

Morphometric measure and meristic study

The specimens were brought out of the cool box and the body length was measured using a one-meter measuring board graduated in millimetres (mm). The morphometric parameters were measured from left side of each specimen. According to Beckman (1962) and Coad (2010, 2017), morphometric characters and meristic were studied as shown in *Table 1*.

Extraction of DNA

Samples were taken from the liver of two fish species their identification by morphological characters only. Samples (20 mg) collected from the liver tissue samples were digested and homogenized, and stored in liquid nitrogen. According the protocol of AccuPrep® Genomic DNA extraction Kit (Bioneer Corporation Cat. No.: K-3032 Korea), genomic DNA was extracted.

Agarose gel (1%) electrophoresis used to assess and identify the quality of the extracted DNA.

PCR amplification

In *Capoeta trutta* to amplify mtDNA COI locus by Darabi (2014) viz., the following primer was used COI-625F: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and COI-625 R: 5'-GACTTCTGGGTGGCCAAA-GAATCA-3' have been used in this study. Amplifications of DNA were performed using a thermal cycler (MultiGene OptiMax Thermal Cycler TC9610 /TC9610-230, Applied Bio systems, USA) with the

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final reaction volume of 25 µl. Each reaction contained prime *taq* premix (2X) Genet Bio PCR master mix (*Taq* DNA Polymerase 1 unit/10 µl, 20 mM Tris-HCl, 80 mM KCl, 4 mM MgCl2, enzyme stabilizer, sediment, loading dye, pH 9.0, 0.5 mM of each dATP, dCTP, dGTP, dTTP), primers (10 pmoles/µl), DNA template (40 ng) and water free DNase. Initial denaturation was carried out for 3 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s; annealing at 62 °C for 30 s and extension at 72 °C for 45 s followed by a final extension at 72 °C for 10 min (Parmaksiz and Eksi, 2017).

Table 1. *Morphological characters and meristic abbreviations and description*

Abbreviations	Description
TL	Total length
SL	Standard length
HL	Head length
BD	Body depth
ED	Eye diameter
SnL	Snout length
Pre-O	Pre orbital distance
PrD	Pre dorsal fin distance
LD	Length of the dorsal-fin ray
Pre-Pectoral	Pre pectoral fin distance
Pre-Pelv	Pre pelvic fin distance
Pre-ans	Pre – anal distance
LA	Length of the anal-fin ray
ALL	Above lateral line scales
BLL	Below lateral line scales
PrD1	First pre dorsal fin distance
PrD2	Second pre dorsal fin distance

Primers have been used to amplify 61 cytochrome b (cytb) gene, partial cds; mitochondrial, primers designed from cytochrome b (cytb) gene GenBank: (JQ060190.1), F: CTGCATTCGTAGGCTATGTC and R: GTGCTAGAACCCCTCCTAGC for *Planiliza abu* fish. PCR was performed with a profile of initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for 30 s, extension at 72 °C for 30 s and final extension at 72 °C for10 min.

Agarose gel electrophoresis separation

A volume of 10 µl PCR product on 2% agarose gel was electrophoresed. Ethidium bromide was used to stain bands, which were visualized on a gel documentation (ENDURO™ GDS Touch Gel Documentation System) by using 100 bp DNA ladder (gene direx). The ladder was supplied in a ready for using format having fluorescent tracking dyes and DNA stain. The expected size of the PCR amplicon was 625 bp for *Capoeta trutta* and 521 bp for *Planiliza abu*.

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DNA sequencing

A mitochondrial DNA cytochrome c oxidase subunit I (mtDNA *COI*) locus and cytochrome b (cytb) gene were amplified by PCR. In the present study, Genetic analyzer 3500, Applied Bio systems (USA) was used to find the nucleotides order of mtDNA *COI* and cytb for *C. trutta* and *P. abu* fish samples, respectively. The PCR product of the fish samples were used for sequence specific PCR amplification and sent to the Macrogen Company in South Korea for nucleotide sequence analyses.

Results

A total of 150 *Capoeta trutta* specimens were collected from Dukan Lake in north western Sulaimani governorate and 120 specimens of the *Planiliza abu* were collected from Sirwan River in south eastern Sulaimani governorate.

Morphology

The morphological characters and meristic of *Capoeta trutta* and *Planiliza abu* are indicated in *Table 2*. *Figures 2* and *3* show the general morphology of *Capoeta trutta* and *Planiliza abu*, respectively.

Table 2. *The results of morphological characters and meristic for Capoeta trutta (Heckel, 1843) and Planiliza abu (Heckel, 1843)*

Abbreviations	Range *Capoeta trutta*	Range *Planiliza abu*
TL	18-32 (25) cm.	14 - 21 (17.5) cm.
SL	15 -27.5 (21.25) cm.	12 -18 (15) cm.
HL	4-5 (4.5) cm	3 – 3.5 (3.25) cm
BD	4.5-7 (5.75) cm	4–5.5 (4.75) cm
ED	0.7 – 1.3(1) cm	0.8 – 0.9 (0.85) cm
SnL	0.9 – 1.5 (1.2) cm	1 cm
Pre-O	1.5-2 (1.75) cm	1 – 1.5 (1.25) cm
PrD	8 – 12.5 (10.25)	-
LD	3.2 – 5.5 (4.35) cm	2.5–3.5 (3) cm
Pre-Pectoral	4 – 5.5 (4.75) cm	4 – 4.5 (4.25) cm
Pre-Pelv	9.5 – 13 (11.25) cm	6 – 7 (6.5) cm
Pre-ans	14 – 20 (17) cm	11.5 – 13 (12.25) cm
LA	3-5 (4) cm	2 – 3 (2.5) cm
ALL	14-17	7
BLL	11-14	6
Number of barbels	One pair barbels on the upper jaw	No barbels
The pectoral fin ray length	3 – 3.8 (3.4) cm	2 – 2.8 (2.4) cm
PrD1	-	7 – 8 (7.5) cm
PrD2	-	11.5 – 13 (12.25)

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Figure 2. *Capoeta trutta*

Figure 3. *Planiliza abu*

DNA sequence

DNA extraction performed on 150 and 120 specimens for *Capoeta trutta* and *Planiliza abu,* respectively were successfully generated product containing DNA.

The result of the present study of sequencing DNA of two species of fish, were mtDNA *COI* locus in *Capoeta trutta* about 617 bp and cytochrome b (cytb) gene, partial cds; mitochondrial in *Planiliza abu* about 446 bp put to BLAST then compared with sequences of other genera and fish species stored in GenBank. The molecular study showed the presence of two species belonging to *Capoeta trutta* and *Planiliza abu*. BLAST results are indicated in *Table 3*.

Table 3. *The BLAST results of fish species*

No. Samples	Genus and species	Molecular based homology (%)
1	*Capoeta trutta*	100% identified homology
2	*Planiliza abu*	100% identified homology

Partial cds, Cytochrome oxidase subunit I (*COI*) gene mitochondrial and partial cds 61 cytochrome b (cytb) gene mitochondrial are compatible with the same sequence fragment marker, which is available at the GeneBank in the National Center for Biotechnology Information (NCBI). *Figures 4a, b* and *5a, b* showed pair wise analysis and partial sequence of the two fish specimens.

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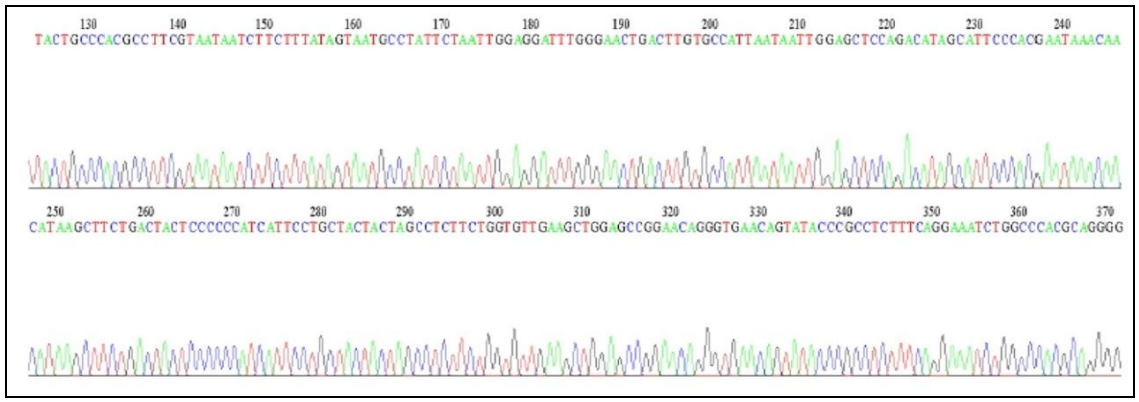

Figure 4a. *The partial sequencing result of partial cds, Cytochrome oxidase subunit I (COI) gene; of Capoeta trutta*

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Query  37   TGGGCACTGCTTTAAGCCTTCTCATTCGAGCCGAATTAAGCCAACCCGGATCACTTCTAG  96
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  29   TGGGCACTGCTTTAAGCCTTCTCATTCGAGCCGAATTAAGCCAACCCGGATCACTTCTAG  88
Query  97   GCGATGACCAAATTTATAATGTTATCGTTACTGCCCACGCCTTCGTAATAATCTTCTTTA  156
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89   GCGATGACCAAATTTATAATGTTATCGTTACTGCCCACGCCTTCGTAATAATCTTCTTTA  148
Query  157  TAGTAATGCCTATTCTAATTGGAGGATTTGGGAACTGACTTGTGCCATTAATAATTGGAG  216
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  TAGTAATGCCTATTCTAATTGGAGGATTTGGGAACTGACTTGTGCCATTAATAATTGGAG  208
Query  217  CTCCAGACATAGCATTCCCACGAATAAACAACATAAGCTTCTGACTACTCCCCCCATCAT  276
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  CTCCAGACATAGCATTCCCACGAATAAACAACATAAGCTTCTGACTACTCCCCCCATCAT  268
Query  277  TCCTGCTACTACTAGCCTCTTCTGGTGTTGAAGCTGGAGCCGGAACAGGGTGAACAGTAT  336
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  TCCTGCTACTACTAGCCTCTTCTGGTGTTGAAGCTGGAGCCGGAACAGGGTGAACAGTAT  328
Query  337  ACCCGCCTCTTTCAGGAAATCTGGCCCACGCAGGGGCATCAGTAGACCTAACAATCTTCT  396
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  ACCCGCCTCTTTCAGGAAATCTGGCCCACGCAGGGGCATCAGTAGACCTAACAATCTTCT  388
Query  397  CACTCCATCTGGCAGGTGTTTCATCAATCCTGGGAGCAATCAATTTCATTACTACAACTA  456
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  CACTCCATCTGGCAGGTGTTTCATCAATCCTGGGAGCAATCAATTTCATTACTACAACTA  448
Query  457  TTAACATAAAACCCCCAGCCATTTCCCAATATCAAACACCCCTATTCGTCTGATCCGTGC  516
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  449  TTAACATAAAACCCCCAGCCATTTCCCAATATCAAACACCCCTATTCGTCTGATCCGTGC  508
Query  517  TCGTAACCGCCGTGTTACTTCTTCTGTCACTACCCGTTCTAGCCGCTGGGATTACAATAC  576
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  TCGTAACCGCCGTGTTACTTCTTCTGTCACTACCCGTTCTAGCCGCTGGGATTACAATAC  568
Query  577  TCCTAACAGACCGAAACCTCAACACCACATTCTTTGACCCCGCCGGAGGAGGAGACCCAA  636
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  TCCTAACAGACCGAAACCTCAACACCACATTCTTTGACCCCGCCGGAGGAGGAGACCCAA  628
Query  637  TCCTCTACCAACACCTA  653
            |||||||||||||||||
Sbjct  629  TCCTCTACCAACACCTA  645
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Figure 4b. *Pair wise alignment partial cds, Cytochrome oxidase subunit I (COI) gene of Capoeta trutta. Query is the study or sample sequence and Subject is the GenBank sequence*

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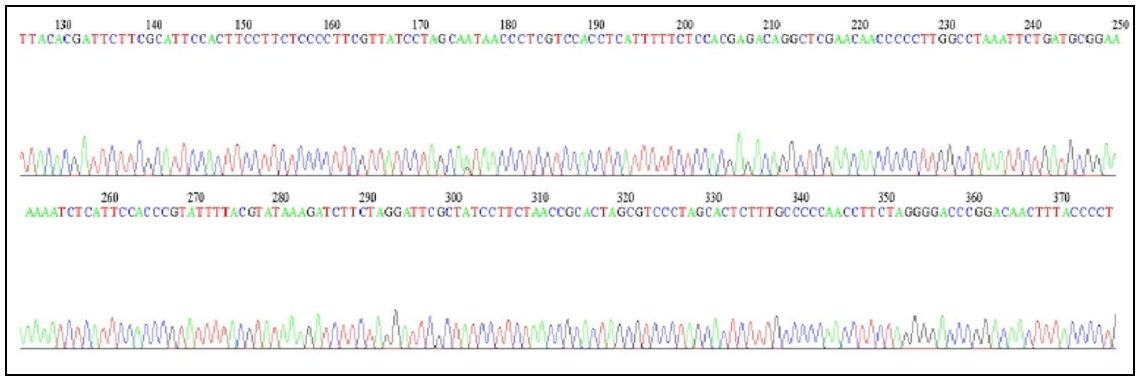

Figure 5a. *The partial sequencing result of Cytochrome oxidase subunit I (COI) gene, of Planiliza abu*

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Query  27   GCGCCACCGTCATTACAAACCTCCTCTCTGCTGTTCCTTATATTGGAGACGCCCTTGTCC  86
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  GCGCCACCGTCATTACAAACCTCCTCTCTGCTGTTCCTTATATTGGAGACGCCCTTGTCC  388
Query  87   AATGAATTTGAGGCGGCTTCTCAGTAGATAATGCTACCCTTACACGATTCTTCGCATTCC  146
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  AATGAATTTGAGGCGGCTTCTCAGTAGATAATGCTACCCTTACACGATTCTTCGCATTCC  448
Query  147  ACTTCCTTCTCCCCTTCGTTATCCTAGCAATAACCCTCGTCCACCTCATTTTTCTCCACG  206
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  449  ACTTCCTTCTCCCCTTCGTTATCCTAGCAATAACCCTCGTCCACCTCATTTTTCTCCACG  508
Query  207  AGACAGGCTCGAACAACCCCCTTGGCCTAAATTCTGATGCGGAAAAAATCTCATTCCACC  266
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  AGACAGGCTCGAACAACCCCCTTGGCCTAAATTCTGATGCGGAAAAAATCTCATTCCACC  568
Query  267  CGTATTTTACGTATAAAGATCTTCTAGGATTCGCTATCCTTCTAACCGCACTAGCGTCCC  326
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  CGTATTTTACGTATAAAGATCTTCTAGGATTCGCTATCCTTCTAACCGCACTAGCGTCCC  628
Query  327  TAGCACTCTTTGCCCCCAACCTTCTAGGGGACCCGGACAACTTTACCCCTGCAAACCCCC  386
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  TAGCACTCTTTGCCCCCAACCTTCTAGGGGACCCGGACAACTTTACCCCTGCAAACCCCC  688
Query  387  TAGTCACCCCACCCCACATCAAGCCCGAATGATATTTCCTCTTTGCATACGCTATTCTCC  446
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  TAGTCACCCCACCCCACATCAAGCCCGAATGATATTTCCTCTTTGCATACGCTATTCTCC  748
Query  447  GCTCCATCCCCAACAAGCTAGGAGGG  472
            |||||||||||||||||||||||||
Sbjct  749  GCTCCATCCCCAACAAGCTAGGAGGG  774
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Figure 5b. *Pair wise alignment of partial cds, Cytochrome oxidase subunit I (COI) gene of Planiliza abu. Query is the study or sample sequence and subject is the GenBank sequence*

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Discussion

Traditionally, in the freshwaters of Iraq, four species (*Capoeta aculeata*, *Capoeta barroisi*, *Capoeta damascina* and *Capoeta trutta*) represent the genus *Capoeta* and four species (*Liza abu*, *Liza klunzingeri*, *Liza oligolepis* and *Liza subviridis*) represent the genus *Liza* (Coad, 2010).

The presences of two species of fish in this study are showed belonging to the family of Cyprinidae and Mugilidae. The description and measurement of present samples of two fish species in this study are similar to those received from Beckman (1962) and Coad (2010).

As a comparison between some characteristics of *Capoeta trutta* in this study such as standard length, total length, body depth, head length, eye diameter, length of the dorsal-fin ray, snout length, length of the anal-fin ray, above lateral line scales and below lateral line scales are 25, 21.25, 4.5, 5.75, 1, 1.2, 4.35, 4, (14-17) and (11-14), respectively are in agreement with the results of Agha (2017), which are 28.18, 23.74, 4.86, 5.7, 0.76, 1.5, 4.98, 3.6, (15-16) and (10-11).

Standard length, total length, body depth, head length, eye diameter, length of the dorsal-fin ray, snout length, length of the anal-fin ray, above lateral line scales and below lateral line scales of *Planiliza abu* in this study are 17.5, 15, 3.25, 4.75, 0.85, 1, 7 and 6, respectively are in agreement with the results of Agha (2017), which are 18.38, 16, 3.53, 4.45, 0.75, 1, 7, 6.

The morphological results of *Planiliza abu* in this study are similar to the results of Khayyami et al. (2014) on morphological variability of *Liza abu* and Mohamed et al. (2016) on comparative taxonomical for (*Planiliza subviridis*, *P. klunzingeri*, *P. carinata* and *Osteomugil speigleri*). Standard length of *Planiliza abu* in this study is in agreement with the results of Mohamed et al. (2018)**.**

In Iraq, there has not been any molecular research on *Capoeta trutta* and *Planiliza abu*, but Cyprinidae and Mugilidae families have been examined. The results of *Capoeta trutta* in family Cyprinidae in this study are in agreement with the results of Faddagh et al. (2012a) who identified eight cyprinid fish species, and discovered high similarity between *Barbus* species, from 84.4% between *B. Kersin* and *B. xanthopterus* to 52% between *B. sharpeyi* and *B. barbulus* to 86.9% between *A. vorax* and *B. grypus.* Faddagh et al. (2012b) also used the mitochondrial 16S rRNA gene fragment as a molecular marker to study taxonomical status of seven cyprinin fish species in Iraqi inland waters: *Barbus kersin*, *B. xanthopterus*, *B. barbulus*, *B. sharpeyi*, *B. grypus*, *Cyprinus carpio* and *C. luteus,* the results assured that the six *Barbus* species genetically belong to sub-family Cyprininae which belong to family Cyprinidae. Aziz (2015) examined nine species of Cyprinidae family, the result of DNA sequencing showed that all species belong to family Cyprinidae the phylogenetic relationship degree with this family for *C. luteus* was a BP of 87%, for *C. regium*, *C. carpio* and *C. Carassius* was a BP of 75%, for *C. macrostomum*, *L. esocinus*, *C. trutta* and *L. xanthopterus*a was a BP of 90% and for *Barbus grypus* was a BP of 76%.

In this study the results are in agreement with Parmaksiz and Eksi (2017) who used mtDNA *COI* 625 locus to study the genetic diversity of populations from 47 samples of *Capoeta trutta.* The result of sequence analysis showed six polymorphic sites and seven haplotypes on that locus, which is also in agreement with Turan (2008) determination of subspecies of *Capoeta* corresponding to taxonomic entities and defined species using traditional gene sequencing of mitochondrial 16S rDNA. The database included 124 variable sites, parsimony informative was 103 sites. The results in this study are similar

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to the results of Zareian et al. (2016) who used mitochondrial cytochrome *b* gene sequences for phylogenetic relationship of *Capoeta* species, and it was found that three major groups were detected: Clade I: *Capoeta trutta* group which is the Mesopotamian *Capoeta* group having very close related taxa (*barroisi*, *trutta and turani)*. Clade II: *Capoeta damascina* complex group (*capoeta* group small scale) including the Anatolian-Iranian groups such us (*buhsei*, *saadii*, *banarescui* and *damascina*) and widespread highly diversified groups. Clade III comprises closely related taxa; *Capoeta capoeta* complex group (the Aralo-Caspian group, large scale *capoeta* group).

The results in this study are in agreement with Nematzadeh et al. (2013) using PCR-sequencing method to establish phylogenetic relationships among six mugilidae species (*M. capito*, *Valamugil buchanani*, *Mugil cephalus*, *Liza subviridis*, *L. saliens* and *L. aurata)* and genetic differences were determined. The results demonstrate that in the mitochondrial 16s rRNA genome number of bases was approximated 600 base pairs. Also (Lai et al., 2011) (80) random primers for random amplified polymorphic DNA (RAPD) were used for the examination of 15 fish families. Results clarify that in the Mugilidae family a novel specific PCR product was found, OPAV04 primer was employed also in the *Liza* genus, by using OPAV10 primer other novel specific PCR product was found.

The results of the present study are not in agreement with Faddagh et al. (2012b the *Liza abu* and *Liza klunzingeri* did not respond to the modified primer in mitochondrial 16S rRNA gene but in this study *Planiliza abu* responded to the cytochrome b (cytb) gene, partial cds; mitochondrial.

This taxonomic position has changed and most researchers in the field now agree that DNA coding is a useful tool in the process of identifying and indexing species. There are still researchers who doubt that one can distinguish the gene of all species and refers to the fact that taxonomists who evaluate their findings on morphological basis have a range of many different characters, not one, to help them identify, for this the present study used a molecular tool for identification. Molecular techniques such as PCR and DNA sequencing were proven to be very specific and highly sensitive to detect species of fish. However, using them in diagnostic laboratories are very rare.. Moreover, DNA amplification is not cheap and it is tedious, also samples can face cross contamination which is dangerous, fortunately nowadays by developed methods these issues are decreased (Agha, 2017).

In the present study, 617 and 446 bp were aligned for *Capoeta trutta* and *Planiliza abu* respectively; the two specimens were morphologically identified by using Coad keys. The sequences compared with sequences of other genera and fish species stored in Gen Bank. The results showed that the morphometric data and molecular methods were successful in identifying of *C. trutta* and *P. abu*.

Samples of *Capoeta trutta* and *Planiliza abu* have been morphologically identified. DNA sequencing results showed that the studied two fish species belong to *Capoeta trutta* and *Planiliza abu.* Gen Bank analysis indicated that the two sequenced species were correctly identified.

Conclusions

In the view of the results of the present study, *Capoeta trutta* and *Planiliza abu* were morphologically identified. The results of DNA sequencing revealed and confirmed the

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validity of the two fish species and indicated that the two sequenced species were correctly identified by using *COI* and cytochrom b gene.

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